Appendix P

In Vitro Cytotoxicity Test Methods and the High Production Volume (HPV) Challenge Program

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Appendix P-1

Supplemental Acute Toxicity Protocol

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U.S. EPA/OPPTS/OPPT/High Production Volume (HPV) Challenge Program

[NOTE: This statement was extracted from the EPA web site. The original can be visited at: http://www.epa.gov/chemrtk/toxprtcl.htm]

Supplemental Acute Toxicity Protocol

The EPA, along with the National Toxicology Program and the National Institute of Environmental Health Sciences (NIEHS), sponsored an International Workshop on *In Vitro* Methods held on October 17-20, 2000, to review the validation status of available *in vitro* methods for predicting acute oral toxicity, among other goals.

The October 2000 Workshop concluded that *in vitro* cytotoxicity data could be useful in estimating starting doses for *in vivo* acute toxicity testing, and in this way could also reduce the number of animals used in subsequent *in vivo* tests. The two candidate cytotoxicity tests recommended for use with the regression model for estimating starting doses from *in vitro* cytotoxicity data are neutral red uptake assays using BALB/c 3T3 mouse fibroblasts and normal human keratinocytes. Other cell lines/cells could also be used with the regression model to estimate starting doses, but first the correlation between the *in vitro* test and the *in vivo* test must be established quantitatively. Guidance on these *in vitro* tests, protocols for use of recommended tests, and a reporting template for results of *in vitro* tests are all contained in the **ICCVAM Guidance Document** (2001), which is one of the products of the October Workshop. Further background on the October workshop can be found in the **ICCVAM Workshop Report** (2001).

While the formal request to EPA from NIH that would ask the Agency to accept or reject these protocols has not yet been received (nor have these methods been incorporated in OECD or the EPA acute toxicity test guidelines), the findings of this workshop included a recommendation to all Agencies participating in ICCVAM to consider the use of these *in vitro* cytotoxicity tests as supplements to the current acute oral *in vivo* acute toxicity protocols. These *in vitro* cytotoxicity protocols were recognized earlier in Steven Johnson's letter of October 30, 2001. The *in vitro* tests are supplements to, not

replacements for, the OECD acute toxicity test guideline 425 (known as the Up-and-Down Procedure) which is currently recommended for use in the HPV Challenge Program. The new *in vitro* tests are intended to better estimate the starting doses for new *in vivo* acute oral toxicity studies conducted under the HPV Challenge.

We encourage those participating in the HPV Challenge Program to consider using the recommended *in vitro* tests noted here as a supplemental component in conducting any new *in vivo* acute oral toxicity studies under the HPV Challenge Program, to note the intention to use these protocols in HPV Challenge test plans submitted to EPA, and to summarize the results using the recommended reporting template. This information on the *in vitro* template should accompany results from the *in vivo* acute oral tests, and be provided to EPA as part of the HPV Challenge Program. The October workshop documents and the recommended reporting template for the *in vitro* tests can be found below. The ICCVAM website - *In Vitro* methods page - should be consulted for any future updates to the *in vitro* guidance methodologies prior to proceeding with testing.

In order to gain more familiarity with these methods, technical experts from industry and other organizations were invited to a workshop sponsored by EPA, NIEHS, and others on these *in vitro* methods. The workshop was held February 19-21, 2001 (see the ICCVAM website at http://iccvam.niehs.nih.gov/meetings/schedule.htm for more details).

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)
Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. 2001. NIH Publication No. 01-4499. National Institute of Environmental Health Sciences (NIEHS)

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods)

Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity. 2001. NIH Publication No. 01-4500. National Institute of Environmental Health Sciences (NIEHS)

Standard Test Reporting Template

Any updates to this methodology can be found under ***In Vitro* Methods on the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) web site.**

Last updated on September 16, 2002

Visit the ICCVAM Home Page

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Appendix P-2

Office of Pollution Prevention and Toxics (OPPT)

Letters to Manufacturers/Importers

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Office of Pollution Prevention And Toxics

Letters to Manufacturers/Importers

[High Production Volume Voluntary Challenge Program]

October 14, 1999
Company name
Street #
City, State, Zip

Dear Company Contact:

On behalf of the Environmental Protection Agency (EPA), I would like to thank you for your commitment to participate in the voluntary High Production Volume Challenge (HPV) program. We look forward to working with you over the coming years as we achieve our goals for this important program.

As you may be aware, a number of animal protection organizations and the public have raised concerns that the HPV Challenge program may lead to the excessive use of animals in tests and to inadequate attention to existing information and alternative testing methods that do not require animals as test subjects. As a general matter, animal experiments should not be performed if another validated method -- not involving the use of animals -- is reasonably and practically available for use in the HPV Challenge program. To respond to these concerns, and after consultation with the organizations involved in developing the framework for this initiative, I am asking you and your fellow HPV Challenge participants to observe the following principles as we proceed with the program:

1. In analyzing the adequacy of existing data, participants shall conduct a thoughtful, qualitative analysis rather than use a rote checklist approach. Participants may conclude that there is sufficient data, given the totality of what is known about a chemical, including human experience, that certain endpoints need not be tested.

2. Participants shall maximize the use of existing and scientifically adequate data to minimize further testing. To reinforce this approach, EPA will consider information contained in the databases identified in the enclosure, or in databases maintained by the organizations identified in the enclosure, to have been known to the Agency within the meaning of Section 8(e) of the Toxic Substances Control Act (TSCA), 42 U.S.C. 2607(e). This policy is limited to information reported by participants under the HPV Challenge program and generated for or contained in these databases as of the date of this letter. In addition, any other potential liability under TSCA Section 8(e) for existing data on HPV Challenge program chemicals will be limited according to the terms of the “Registration

Agreement for TSCA Section 8(e) Compliance Audit Program (56 Fed. Reg. 4128, Feb. 1, 1991)." This policy does not affect prior 8(e) enforcement actions.

3. Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships.

4. Consistent with the Screening Information Data Set (SIDS) program of the Organization for Economic Cooperation and Development (OECD), participants shall not conduct any terrestrial toxicity testing.

5. Participants are encouraged to use in vitro genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use.

6. Consistent with the OECD/SIDS program, participants generally should not develop any new dermal toxicity data.

7. Participants shall not develop sub-chronic or reproductive toxicity data for the HPV chemicals that are solely closed system intermediates, as defined by the OECD/SIDS guidelines.

8. In analyzing the adequacy of screening data for chemicals that are substances Generally Recognized as Safe (GRAS) for a particular use by the Food and Drug Administration (FDA), participants should consider all relevant and available information supporting the FDA's conclusions. Participants reviewing the adequacy of existing data for these chemicals should specifically consider whether the information available makes it unnecessary to proceed with further testing involving animals. As with all chemicals, before generating new information, participants should further consider whether any additional information obtained would be useful or relevant.

9. Because validated non-animal tests for some SIDS endpoints may be available soon, participants shall make the following revisions to the sequence of testing:

(a) Testing of closed system intermediates, which present less risk of exposure, shall be deferred until 2003;

(b) Individual chemicals (i.e., those HPV chemicals not proposed for testing in a category) that require further testing on animals shall be deferred until November 2001.

These revisions should not be construed to suggest that delay or deferral is appropriate with respect to testing of scientifically appropriate categories of related chemicals.

10. Companies shall allow 120 days between the posting of test plans and the implementation of any testing plans.

ENCLOSURE A

The IUCLID database administered by the European Union's Existing Chemicals Bureau
Aquatic Information Retrieval (AQUIRE)
Catalog of Teratogenic Agents (CTA)
Chemical Carcinogenesis Research Information System (CCRIS)
Chemical Information System (CIS)
The ChemID database of the National Library of Medicine (NLM)
Datalog
Developmental and Reproductive Technology (DART)
Envirofate Environmental Mutagen Information Center (EMIC)
Environmental Teratology Information Center (ETIC/ETICBACK)
GENE-TOX
Hazardous Substances Data Bank (HSDB)
Integrated Risk Management System (IRIS)
Merck Index National Institute for Occupational Safety and Health (NIOSH)
National Library of Medicine TOXLINE and TOXNET
National Toxicology Program (NTP) Testing Information and Study Results
NTP Technical Reports
NTP Chemical Health and Safety Data
Phytotox Registry of Toxic Effects of Chemical Substances
Structure and Nomenclature Search System (SANSS)
Toxics Substances Control Act Test Submissions (TSCATS)
WHO/IPCS Documents (CICADS and Environmental Health Criteria Documents) BIODEG
BIOLOG
CANCERLIT
CHEMFATE
CHRIS
FIFRA Database/MRID
IRAC Documents
MEDLINE
National Cancer Institute Journal
POISINDEX
Shepard's Catalog
STN (Chemical Abstracts Service)

Document Source: http://www.epa.gov/opptintr/chemrtk/ceoltr.htm

To promote the availability and use of alternatives to tests involving animals, the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP) will commit at least $1.5 million in FY 2000, and $3 Million in FY 2001, and any further funds appropriated by Congress, to the development and validation of non-animal alternative test methods and protocols. EPA will provide an additional $250,000 this year and will seek to provide a similar amount next year to these efforts. The Multicenter Evaluation of In Vitro Cytotoxicity (MEIC), on the agenda for the October 14 meeting of NTP's Advisory Committee on Alternative Toxicological Methods, will be given priority attention. EPA will promptly incorporate, as appropriate, the work of NIEHS and NTP into the HPV Challenge program.

EPA recognizes that the HPV Challenge is a voluntary program that includes substantial public review and involvement. The successful implementation of the changes described in this letter will depend upon the good faith effort and cooperation of all parties. We appreciate the spirit of cooperation and commitment that has characterized this initiative to date. The changes to the HPV Challenge program outlined above present the opportunity to advance our shared goals of expanding the basic health data available to the public, while incorporating certain animal welfare concerns and scientific principles. It is the intention of the Agency that the HPV Challenge program, including the test rule(s), should proceed in a manner that is consistent with these principles and concerns.

Again, I thank you for your commitment to participate in the HPV Challenge program. If you need further clarification or assistance with this program, please contact Barbara Leczynski at 202-260-3749 or visit the website at www.epa.gov/chemrtk.

Sincerely,
/s/
Susan H. Wayland
Deputy Assistant Administrator

Enclosure